WATER-BORNE MICRO-ORGANISM CRYPTOSPORIDIUM DETECTION USING IMAGE PROCESSING

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Abstract: The work presented in this paper uses a novel approach into the detection and identification of Cryptosporidium oocysts, based on Machine Vision technology applied to drinking water. Our new concept of cryptosporidium detection uses image processing which allows detailed inspection of parasite morphology to nanometer dimensions making the detection more reliable than existing manual methods. Combining Normarski Differential Interface Contras (DIC) and florescence microscopy using FITC and UV filters the system provides a reliable detection of Cryptosporidium with a considerable reduction in time, cost and subjectivity over the current labour intensive time consuming manual method.

Introduction

Cryptosporidium in drinking water has been widely recognized as a serious cause of concern, with a very large number of waterborne infections caused by its oocysts. In its transmissive stage – the oocyst - is a frequent inhabitant of raw water sources used for the abstraction of potable water. The problem is increased because a small dose can produce cryptopsporidiosis and conventional water treatment process, including chemical disinfection, cannot guarantee to remove or destroy oocysts completely.

In the last decade over half million people has been infected [1] in the UK and US in over 20 well documented water borne outbreaks [2]. In Asia, Africa and Latin America there is not completed statistics, but it is estimated that million of people has been affected with some fatal consequence in the infant population.

Regulatory bodies from all over the world acknowledge the continuous monitoring of water sources for Cryptosporidium as imperative. Many requirements, rules and regulations are in place to attempt to address the control of Cryptosporidium which threatens the safety of drinking water. The current European Union drinking water directive [3] requires that the drinking water is free from micro-organisms to a certain level that does not constitute a potential danger to human health.

In UK in 1999 the Secretary of State for the Environment, Transport and Regions has issued an amendment to "The Water Supply Regulations" which states that water intended for human consumption should contain no more than one Cryptosporidium oocyst in 10 litres of water [4]

In order to assure the implementation of this regulation, a Standard Operating Protocol (SOP) for the Monitoring of Cryptosporidium in Treated Water Supply has been issued [4]. The main tasks covered by this protocol are briefly presented as a diagram in figure 1.

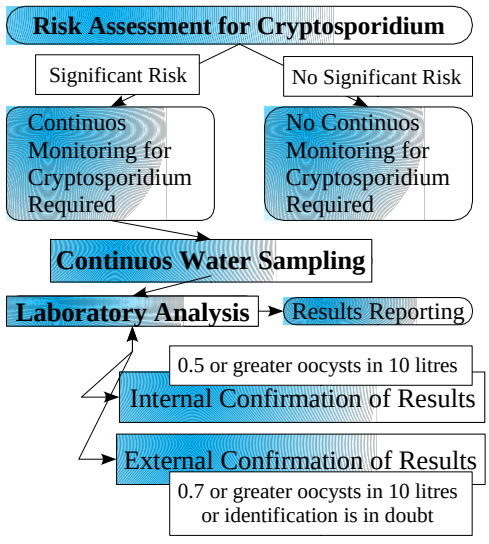

Figure 1: Standard Operating Protocol for the Monitoring of Cryptosporidium oocysts.

The Machine Vision System developed by Glasgow Caledonian University in cooperation with the Scottish Parasite Diagnostic Laboratory and the Technical

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University of Cluj Napoca has been designed and implemented to simplify and improve the detection and analysis procedures required by the SOP (Laboratory and Analytical Procedures).

The Cryptosporidium oocyst structure is shown in figure 2. The three microscopy techniques used are Normarski Differential Interference Contrast (DIC) microscopy, Fluorescent using FITC filters and Fluoresce using UV filters [5].

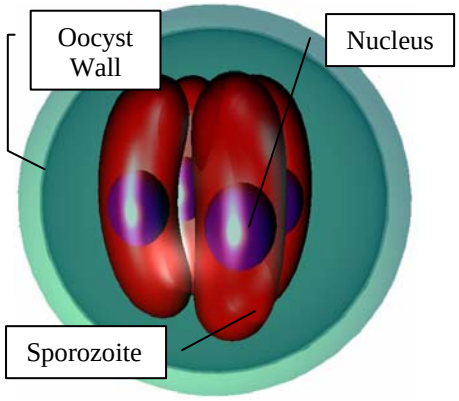

Figure 2: Computer generated model of Cryptosporidium

Materials and Methods

Detection of Cryptosporidium oocysts using DIC microscopy is a tedious and time-consuming procedure required to assess Cryptosporidium presence is the manual microscopic examination of the recovered deposit for the detection and enumeration of Cryptosporidium oocysts. Following the laboratory preparation, each slide well must be scanned in a systematic fashion, ensuring that no field of view is missed and no duplicate counting occurs.

This method is not used under the current SOP because it is very unreliable due to the fact that under DIC microscopy the contrast between the object of interest and the background is minimal; therefore objects are very difficult to distinguish by a human operator. Also under DIC microscopy it is impossible to see the nucleus inside sporozoites; hence sporozoites detection is based only on the shape deformation of the of the external oocyst wall – a very subjective process. Therefore accurate Cryptosporidium identification is impossible using DIC.

Our unexplored system uses the concept of making Cryptosporidium detection under DIC microscopy more reliable using Machine Vision Technology. Cryptosporidium identification was not addressed due to the lack of information regarding Cryptosporidium morphology present in the acquired images.

A series of images from a test slide containing Cryptosporidium, viewed under DIC microscopy with a 20x objective (200x total magnification) were acquired as shown in figure 3.

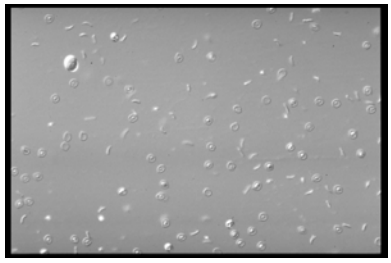

Figure 3: Test slide under DIC microscopy, 200x total magnification.

Results

A colour space conversion (RGB-HSL) is used to extract the light plane, as there is no information associated with colour. Then it is needed to isolate objects of interest from the background. Unfortunately there is no clear separation between pixels belonging to background and pixels belonging to the objects of interest, with the background sliding inside the objects of interest and very little contrast. A LUT transform proved to be completely inefficient in improving the separation, but a significant gain was achieved using a highlight details filter. The result of a highlight details

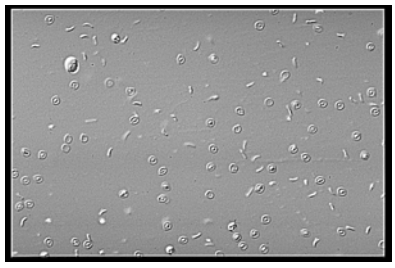

Figure 4: Details Highlighted

filter using a kernel size of 7 is presented in figure 4**.** The separation between the background and the objects

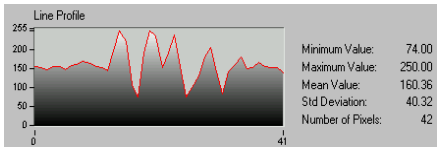

Figure 5 : Cryptosporidium Profile after image processing

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of interest is defined to a certain degree that threshold algorithm can be performed as seen in figure 5.

Objects of interest are constituted from two components, a component lighter than the background and a darker one. The background also slides into the object boundaries. A threshold is performed, in two phases, one for the lighter component, and one for the darker component, then the results are combined using an OR function shown in figure 6.

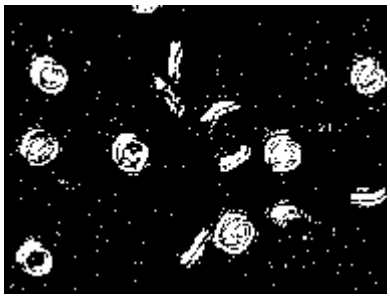

Figure 6: Threshold images combined

It can be noted that the resulting image present prominent binary noise and the objects of interest are incomplete. All objects truncated by the acquisition process, namely objects which are partially in the image and partially outside the image boundaries are eliminated.

The binary noise present is efficiently eliminated using the following algorithm:

1 A buffer copy of the image to be cleaned up is generated.

2 Two successive erode functions are applied on the original image.

3 All pixels from the buffer copy 8-connected to the non-zero pixels from the image are added to the image.

4 Step 3 is repeated until no pixel is added.

The result is presented in figure 7. It can be noted that, unlike other binary noise reduction algorithms, the objects of inters are unaffected.

Figure 7: Binary noise clean-up

The next step is to reinsert the missing pixels within the objects boundaries. A closing algorithm is performed, using a kernel size of 5.

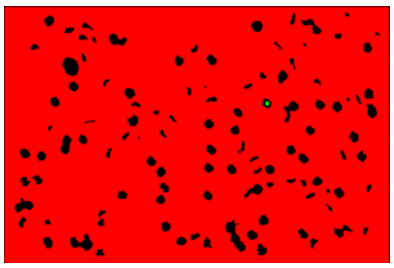

Figure 8: Pseudo-colour display of the labelled negate image

A NOT function is performed, followed by a labelling function. The result is an image which has a value of 0 associated with all objects, a value of 1 associated with the background and a value greater than 1 for every hole in the objects, figure 8. By replacing the values greater than 1 with 0 and negating the image again we achieve holes filling.

All objects too small to be a Cryptosporidium oocyst, therefore not worth analysing are eliminated. This is achieved using the same algorithm as for binary noise removal, but with 7 erosion functions applied.

Then a distance function is applied, and Danielsson [6] circle detection algorithm is used for Cryptosporidium detection. The result is presented in figure 9.

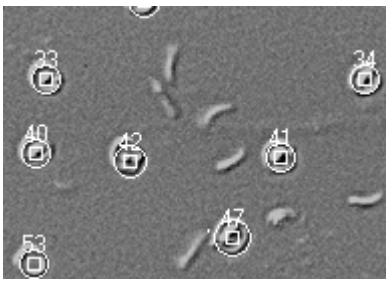

Figure 9: Final result

The presented algorithm is able to detect presumptive Cryptosporidium oocyst on a slide viewed under DIC microscopy. However, from our laboratory test results, the detection rate was 93.75%. Although this is not a disappointing recognition rate, the target detection rate in order for a Machine Vision approach to be used as a standard operating environment must be 100%.

Identification of Cryptosporidium oocysts using exclusively DIC microscopy was considered unreliable therefore not recommended for Cryptosporidium recognition.

Although using DIC microscopy used with image processing dose not provide a 100% detection of cryptosporidium, it can be used as a first stage of the

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new system proposed to provide a reliable detection which combining it with fluorescent microscopy. The new vision system proposed has designed to implement the requirement of "Standard Operating Protocol for the Monitoring of Cryptosporidium Oocysts in Treated Water Supply"[7].

One aim is to include as much as possible the human expertise accumulated at Scottish Parasite Diagnostic Laboratory in the identification of Cryptosporidium to provide a reliable machine vision solution. The system should be able to incorporate current knowledge in the field, but should be kept open for future development, as the knowledge base is increasing over time. Under these circumstances, we decided to match exactly in software the procedure followed by a human operator under current SOP. Our approach lends itself to large amount of water monitoring, as required by an industrial process.

The current process of Cryptosporidium identification is based on a series of Colour Feature Extraction and Shape Feature Extraction under FITC and DAPI, at 1000x total magnification. Therefore for each object to be analysed two images are acquired and processed. This is a time consuming process, during which the sample has to be exposed to UV light. Our solution to overcome this problem was to develop an algorithm that quickly detects relevant objects which resembles Cryptosporidium, objects to which we refer as "Potential Cryptosporidium Oocysts". This algorithm is called the Detection Algorithm, and works as the first stage in the sample analysis process, using a total magnification of 200x and FITC filters. Each potential Cryptosporidium oocyst (defined as roughly circular objects within Cryptosporidium size) is detected and tagged (to avoid double registering of an oocyst due to positioning errors), and its position is passed to the second stage – Advanced Analysis. An accurate map preview of the position of the potential Cryptosporidium oocysts is generated.

Detection of FITC stained Cryptosporidium using Epi-illumination fluorescence microscopy is considered the best method available, being the one employed in the current Standard Operating Protocol.

It provides higher contrast between the objects of interest and background, but samples have to be chemically processed prior to examination. These methods are tedious and time-consuming.

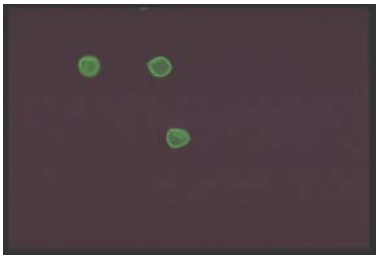

Figure 10: FITC stained Cryptosporidium under Epi-illumination fluorescence microscopy

An image of a slide containing FITC stained Cryptosporidium, acquired using Epi-illumination fluorescence microscopy is presented in figure 10.

Discussion

The excellent contrast between the objects of interest and background provided by this method make thresholding and binarisation possible using a simple clustering, with little noise presented in the binary image. Noise clean-up is performed, followed by objects reconstruction. Then Danielsson Circle detection is employed to perform potential oocyst detection, figure 11. The reason for using a Danielsson circle detection algorithm, instead of just performing morphological analyses for each binary object, is that Cryptosporidium oocyst may be touching each-other, and Danielsson circle detection algorithm is able to detect circular objects touching each-other. The circles with corresponding Cryptosporidium size are defined as suspicious objects detected and their position is recorded for further analysis. In our laboratory test results, detection using this algorithm was 100% successful.

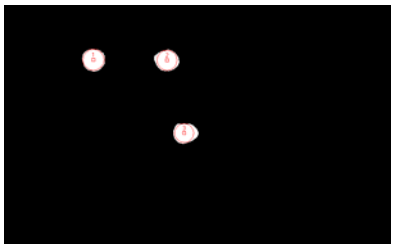

Figure 11: Detected circles

The Advanced Analysis algorithm is a feature extraction algorithm, extracting relevant information under FITC and DAPI filters at 1000x total magnification, for each potential Cryptosporidium oocyst tagged by the Detection Algorithm. These parameters are stored together with the images acquired for each potential Cryptosporidium oocyst for each mode.

After the completion of the Advanced Analysis the system goes into the next stage – Artificial Intelligence Decision Making. This stage is completely transparent to the operator, and uses a fuzzy logic inference engine to mimic human knowledge based decision making. The classification is based on the features extracted by the Advanced Analysis Algorithm and a customisable rule base.

The reliability of the system is dependent on the quality of the acquired images. Therefore algorithms have been developed to assure the proper quality: Auto-focus and Flat Field Correction. A system architecture

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of the whole machine vision system is presented in figure 12.

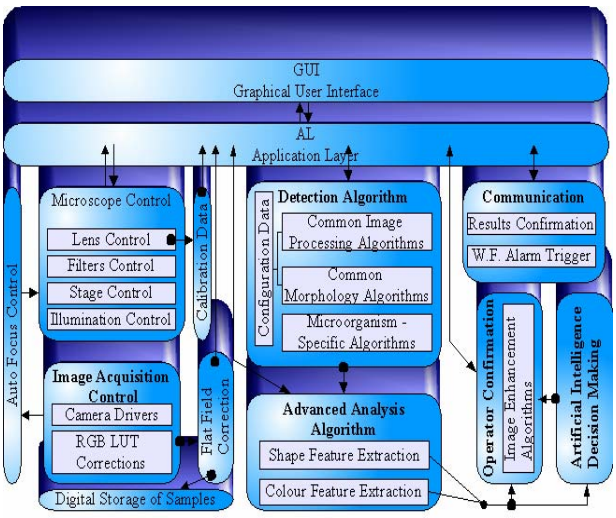

Figure 12: Software Architecture

Conclusions

The Machine Vision approach presented in this paper can perform automated analysis to determine whether or not Cryptosporidium oocysts are present in treated water supply. The system can reliable determine the presence of Cryptosporidium and enable the sample to be accurately and efficiently reviewed by an operator if required.

The novel approach of detection proposed here allows a reliable detection of waterborne micro-organism Cryptosporidium with substantial reduction in process time and cost than the current methods in use and permit the assessment of large quantity of water quality.

The implemented algorithms accommodate feature such as Cryptosporidium size, shape, nucleons number, DAPI internal staining and typical or atypical FITC staining. In order for the system to completely match a highly trained human operator expertise, feature extraction algorithms to match the linguistic terms of suture-line presence and rim fluorescence characteristics must be developed.

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